

US EPA ARCHIVE DOCUMENT



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,  
PESTICIDES AND TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Environmental Hazard Assessment for the Microbial Pesticide,  
*Aspergillus flavus* AF36 for Conditional Registration in Arizona and EUP  
Extension in Texas; **EPA Reg. No.** 071693-R; **Barcode Nos.** D288777;  
D288782; D286708

**FROM:** Gail S. Tomimatsu, Ph.D. *Gail Tomimatsu*  
Microbial Pesticides Branch  
Biopesticides and Pollution Prevention Division, 7511C

**PEER REVIEW:** Zigfridas Vaituzis, Ph.D., Senior Scientist *Zigfridas Vaituzis*  
Microbial Pesticides Branch  
Biopesticides and Pollution Prevention Division, 7511C

**TO:** Shanaz Bacchus, Regulatory Action Leader  
Biopesticides and Pollution Prevention Division 7511C

**Pesticide Name:** *Aspergillus flavus* Strain AF36-colonized wheat seed  
**Registrants:** The Arizona Cotton Research and Protection Council and the U.S. Dept. of  
Agriculture (Agricultural Research Service and IR-4 Programs)

The attached documentation contains the Environmental Hazard Assessment for *Aspergillus flavus* AF36 (AF36) for use as a microbial pesticide to "displace" (or suppress) populations of aflatoxin-producing *A. flavus*, in partial support for FIFRA registration on cotton in Arizona and for extension of the EUP in Texas. Our conclusion of "**reasonable certainty that no incremental hazards to nontarget organisms or to the environment are anticipated as a result of the intended use**" is based on an extensive database, containing data from OPPTS Guideline Studies and the open literature.

Also, Data Evaluation Records (DERs) are attached for the Avian Inhalation Test (MRID #4579102) and for Field Testing of Pollinators, Honeybee (MRID# 45739102). An evaluation of the basic biology and ecology of the endangered species, Plover is included in this assessment also [MRID # 45739103; BPPD Review, Gail Tomimatsu, April 22, 2003]. This assessment considers all DERs, evaluation of plover ecology, previous reviews of 1996, 1999, 2001, and the monitoring and product performance studies (MRID #s 453072-01 and 45307202), reviewed under a separate cover memorandum (BPPD Review, May 15, 2003.).

## ENVIRONMENTAL ASSESSMENT

### I. AF 36 Environmental Hazard Assessment

The environmental hazard (risk) assessment for AF36 is characterized in two portions: **A)** environmental expression and exposure assessment and **B)** nontarget wildlife (fauna and flora) assessment. Specific data are cited relative to *Aspergillus flavus* populations in air and soils in Arizona cotton agroecosystems, effects on non-target avian species (Northern Bobwhite) and honeybees, and endangered or threatened wildlife, particularly avian species. The results of these studies are presented here in both tabular (Table 3) and more detailed descriptive formats. A complete record of the submitted data can be found in the individual Data Evaluation Reports (DERs) and five memoranda [References]. Waiver rationale for testing potential toxicity/pathogenicity effects on other nontarget organisms (i.e., aquatic organisms, plants and insects) were considered also.

#### A. Environmental Expression and Exposure Assessment

Atoxigenic strains of *A. flavus*, such as strain AF36, occur throughout the southern United States as part of the resident microflora in agroecosystems, particularly cotton, corn and peanuts. As a consequence, all terrestrial and aquatic fauna and flora could be exposed to unintended effects of the MPCA. Prior to 2001, little documentation was available to evaluate the ecotoxicology and environmental expression of *A. flavus* Strain AF36, hereafter referred as AF36.

At the request of the Agency, longer term studies were necessary to determine the extent of AF36 colonization and survivability in cotton agroecosystems, particularly at crop maturity (typically in September and October) and in the year(s) following AF36 application. These scientifically valid studies (portions of these studies are in peer-reviewed scientific journals) are summarized below. The extreme variability noted in results of population studies submitted in 1999 [Review of Request for Waiver of Non-Target Organism Safety Effects for EUP Renewal and Expansion for Biopesticide Containing *Aspergillus flavus* AF36, from D. Gurian-Sherman, June 23, 1999] suggested a lack of confidence that these levels do not increase overall *A. flavus* levels at crop maturity, thereby possibly increasing exposures to nontarget wildlife. Also, incremental exposures to AF36, a relative of an opportunistic pathogen, *Aspergillus flavus* (endemic to the Arizona desert ecosystems) were of concern for potential hazards to avian and bee species.

#### 1. Environmental Expression

Air and soil populations of *Aspergillus flavus* and AF36 were monitored for two to three years in several AZ cotton fields. Results of these studies suggested "replacement" of toxigenic (aflatoxin-producing; "S" phenotype) fungi with atoxigenic fungi (L phenotype) without increasing the overall quantity of *A. flavus* in the soil. [MRID #s MRID #s 453072-01, 453072-02 ; BPPD Review of Soil and Air Monitoring Studies and Product Performance Testing (Efficacy) Submitted by USDA Southern Regional Research Center/IR-4 as a Condition of Registration and EUP Extension (Texas) for *Aspergillus flavus* AF36, from Gail Tomimatsu, May 15, 2003]. Results of morphological comparisons and genetic complementations with (vegetative compatibility grouping-VCG), and aflatoxin measurements of several hundred L-phenotype isolates from these

populations demonstrated the “stability” of the atoxigenic phenotype among members of the genetic group of AF36. Furthermore, L-strain isolates of the AF36 VCG (Vegetative Compatibility Group) from treated fields, including fields treated several years previous, do not produce aflatoxins in tests whereas S strain isolates from the same fields produce very large quantities of aflatoxins.

There were no significant differences between the two sites in either the mean number of *A. flavus* propagules  $\text{m}^{-3}$ , nor in total fungal propagules  $\text{m}^{-3}$ . Population counts of *A. flavus* ranged from an average of  $29.7 \text{ cfu m}^{-3}$  (range =  $<1$  to  $406 \text{ cfu m}^{-3}$ ) in the untreated field, to an average of  $28.6 \text{ cfu m}^{-3}$  (range =  $<1$  to  $416 \text{ cfu m}^{-3}$ ) for the treated plot. Peaks in *A. flavus* (and total fungal) propagules coincided with boll maturation and cotton harvest within the area., thereby suggesting that application of the atoxigenic isolate may have altered the composition of *A. flavus* in the air, without altering the total quantity (toxigenic + atoxigenic) *A. flavus* recovered. It is commonly accepted that *A. flavus* is aerially dispersed, and can travel several hundred meters, depending on air currents, speed or other biological dispersal agents, e.g., insects.

Greatest quantities of the aflatoxigenic phenotype occurred between May and August of both years at the untreated site, when compared to the treated site. Furthermore the proportion of the atoxigenic phenotypes did not appear to change significantly between 1997 and 1998, which suggests that the effect of AF36 treatment did not disappear after a single season.

Raw data were summarized in the submitted studies with appropriate references of methodology and materials for sample collection, dilution plating on agar media, strain classification (by vegetative compatibility analysis to identify AF36) and by morphological criteria to separate the S and L strains) and assessment of aflatoxin-producing potential. Where appropriate, weather monitoring data were included also.

## **B. Non Target Wildlife Hazard Assessment**

Based on the evaluation of the submitted maximum hazard dose testing data and the exposure assessment of atoxigenic AF36, no unreasonable adverse effects on the terrestrial and aquatic wildlife of the cotton agroecosystem are expected from aerial, or in-furrow applications of sterile wheat seed colonized with *Aspergillus flavus* AF36.

### **1. Summary of Non-Target Organism Toxicity/Pathogenicity Testing.**

The Agency required avian and honeybee studies to address potential pathogenicity concerns of the MPCA *Aspergillus flavus* AF36; because of its relation to a known opportunistic pathogen, *Aspergillus flavus*. The form of the test substances used in these tests were the TGA1 (Avian Inhalation Testing, OPPTS 885.4100) and the wheat-formulated end use product (Field Testing of Pollinators, OPPTS 850.3040; Honeybee Testing, OPPTS 885.4380). Although other nontarget organism studies were waived in previous memoranda [BPPD Review of Information submitted by USDA Southern Regional Research Center/IR-4 for an Experimental Use Permit for *Aspergillus flavus* AF36, from Gail Tomimatsu, April 24, 1996; Review of Request for Waiver of Non-Target Organism Safety Effects for EUP Renewal and Expansion for Biopesticide Containing

*Aspergillus flavus* AF36, from D. Gurian-Sherman, June 23, 1999]; the Applicants submitted formal requests to waive testing accompanied with relevant scientific rationale.

**Table 3.** Tabular results of non-target wildlife and environmental expression data, with supporting MRIDs

USEPA OPPTS Guideline No. <sup>a</sup>	Study Type	Summary & Results	MRID No(s)
885.4050 154A-16	Avian Oral Toxicity	No incremental hazards of AF36 for avian species are anticipated for this use. Results of soil and air population studies and the avian inhalation test (OPPTS 885.4100), and acceptable waiver rationale support this finding.	43763403, 43763405 45739103, 45307201, 45307202 45798102
885.4100 154A-17	Avian Inhalation Test	No incremental hazards of AF36 for avian species are anticipated for this use. Young bobwhite quail treated with <i>Aspergillus flavus</i> AF36 at a mean daily inhalation dose of $1.44 \times 10^5$ cfu per bird for five consecutive days exhibited no toxic or pathogenic effects during the 30 day observation period.	45798102; 45307202 ,
885.4150 154A-18	Wild Mammal Testing, Tier I	No incremental hazards of AF36 for wild mammalian species are anticipated for this use. Results of soil and air population studies, the acute oral pathogenicity and acute pulmonary pathogenicity tests (OPPTS 885.3050 and 885.3150), and acceptable waiver rationale support this finding.	43763405, 45307201, 45307202 43972403
885.4200 154A-19	Freshwater Fish Testing	No incremental exposures of AF36 for freshwater fish are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> and acceptable waiver rationale support this finding.	43763403 43763405, 45307201, 45307202

USEPA OPPTS Guideline No. <sup>a</sup>	Study Type	Summary & Results	MRID No(s)
885.4240 154A-20	Freshwater Invertebrate Testing	No incremental exposures of AF36 for freshwater aquatic invertebrates are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> and acceptable waiver rationale support this finding	43763403 43763405 45307201 45307202
885.4280 154A-21	Estuarine and Marine Animal testing, Tier I	No incremental exposures of AF36 for estuarine and marine organisms are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> and acceptable waiver rationale support this finding	43763403 43763405 45307201 45307202
885.4300 154A-22	Nontarget Plant Studies, Tier I	No incremental exposures of AF36 for nontarget plants are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> and acceptable waiver rationale support this finding	43763403 43763405 45307201 45307202
885.4380 154A-24 850.3040	Honeybee Testing, Tier I; Field Testing of Pollinators	No incremental hazards of AF36 for honeybees are anticipated for this use. The exposure and potential hazard of <i>Aspergillus flavus</i> AF36 colonized-wheat seed to foraging honey bees ( <i>Apis mellifera</i> L.) on blooming cotton was assessed for 30 days, following an aerial application at label rates. <i>Aspergillus flavus</i> AF36 applied once at 10 lbs/acre was not hazardous to honey bees.	45739102
885.4340 154A-23	Nontarget Insect Testing	No incremental exposures of AF36 for insects are anticipated for this use. Results of soil and air population studies for AF36 and <i>Aspergillus</i> , honeybee field tests (OPPTS 885.4380 and 850.3040) and acceptable waiver rationale support this finding	43763403 43763405 45307201 45307202 45739102

USEPA OPPTS Guideline No. <sup>a</sup>	Study Type	Summary & Results	MRID No(s)
<b>885.4000</b> 154A-1, 2, 3, 4, 5	Background for Microbial Pesticides Testing	Results of multiple year soil and air population monitoring studies which demonstrate that the intended application of the MPCA does not significantly increase the overall quantity of <i>Aspergillus flavus</i> at cotton crop maturity, nor in the soil one year after application.  The results also suggest that the pesticidal mode of action may be attributed to competitive displacement of aflatoxin-producing <i>Aspergillus flavus</i>	45307201, 45307202
<b>885.5000</b> 155A-1, 2	Environmental Expression Testing		
<b>810.1000</b> 90-1, 90-3 90-30	Product Performance Testing		
None	Endangered Species Impact Assessment	No incremental exposures of AF36 for endangered wildlife are anticipated for this use. Soil and air population data for AF36 and <i>Aspergillus</i> support this finding. No incremental hazards of AF36 are anticipated to endangered avian species in Arizona and Texas, due to results from the avian inhalation test (OPPTS 885.4100), and assessments of feeding habits and preferred habitats for birds of prey and insectivorous birds in Arizona, and for plovers in Texas. No incremental hazards of AF36 are anticipated to endangered mammals due to results from acute oral pathogenicity and acute pulmonary pathogenicity tests (OPPTS 885.3050 and 885.3150).	45739103 45307201, 45307202 45798102 45307202 43972403

<sup>a</sup> **OPPTS Guideline Numbers** are bold-faced; Existing OPP Guidelines are normal text.

## 2. Non-target Wildlife Testing and Hazard Assessment

### a. Avian wildlife hazard assessment

In consideration of the exposure assessments gathered to date, and avian toxicology information and data as summarized below, no hazards to wild or domesticated avian species are anticipated as a result of AF36 registration on cotton. Likewise, no incremental hazards are anticipated to endangered avian species, because of lack of exposures to these species (see **Endangered Species Assessment**).

During the EUP registration period of 1996 to 1999, EPA reviewed several studies submitted by the Applicant to justify their requests to waive avian toxicology testing [BPPD Review, April 24, 1996; Review, June 23, 1999], which were based on claims of natural occurrences of AF36, and minimal or no bird usage (=no exposure of birds to AF36-treated wheat seed) of cotton fields. Exposures of *Aspergillus flavus* AF36 to avian wildlife are expected because of their ubiquitous nature [MRID #s: 43763403; 43763405; Review, June 23, 1999]. Portions of the studies demonstrated conclusively bird presence in cotton fields [MRID #s: 44464202; 44452615; and 44477901] at the time of intended application; and the likelihood of exposures to indigenous avian species. Avian pathogenicity testing was required also because of



concerns for avian hazards: *a) A. flavus* grows well at avian body temperatures, and *b) uncertainty* as to its role in aspergillosis in domesticated fowl [BPPD Review, April 24, 1996; Review, June 23, 1999].

#### 1. Avian wildlife exposure

Avian abundance, species composition and use of fields and adjacent habitats were documented for Arizona, Texas, and Alabama/Mississippi. [MRID #s 44464202; 44452615; 44477901; Review, June 23, 1999]. In Arizona, avian use (54 species observed) of cotton fields averaged over 50% (compared to adjacent habitats). The census revealed that a number of indigenous AZ bird species were found in numbers comparable to those in adjacent native habitats and would be coincident with AF-36 treated seed presence in treated cotton fields. Because birds were found to spend a significant amount of their time foraging these areas, it was concluded likely that birds will find, and be exposed to increased levels of AF36-treated wheat seed.

#### 2. Avian toxicology studies

In 1999, EPA determined that an avian inhalation pathogenicity test be performed to assess pathogenicity of AF36 [Review, June 23, 1999]. EPA approved a test protocol [MRID #s 45307201, 45307202; Review of Protocol for Testing the Toxicity/Pathogenicity of the MPCA. *Aspergillus flavus* Strain AF36 to Avian species, from Gail Tomimatsu, June 29, 2001], and determined a maximum hazard dose based upon *A. flavus* population data from air monitoring studies [MRID #s 45307201, 45307202].

An acceptable guideline study {OPPTS 885.4100} demonstrated the potential toxicity of *Aspergillus flavus* AF36 to young bobwhite quail (26 day old) in a maximum hazard dose inhalation study. [MRID 45798102; DER from Gail Tomimatsu, April 16, 2003]. Thirty bobwhite quail were treated with *Aspergillus flavus* AF36 by intratracheal instillation at a mean daily dose of  $1.44 \times 10^5$  cfu per bird for five consecutive days. There were no clinical signs of toxicity and no treatment-related effects evident in body weight change or food consumption during the 30 day observation period following treatment. In addition, there were no abnormalities in macroscopic *post mortem* evaluations. The study complied with Good Laboratory Practices of the United Kingdom and Organization of Economic Cooperation and Development.

No further avian testing is required for *Aspergillus flavus* AF36 for cotton. The inhalation test as conducted is a stringent stress test, compared to testing via the oral route of exposure. Since there were no adverse effects reported in the inhalation pathogenicity study, and since there were no reports of adverse effects in birds under actual field use during an EUP, an oral toxicity/pathogenicity study is not justifiable for this registration.

#### **b. Mammalian Wildlife**

The mammalian toxicology information gathered to date on does not show a hazard to wild or domesticated mammals. The AF36 toxicity data for Human Health Assessment indicate that there is no significant toxicity nor pathogenicity to rodents from acute oral and pulmonary testing at the maximum hazard dose, summarized below. Therefore no incremental hazard to mammalian wildlife is anticipated.

Acute oral studies on laboratory rats indicate no toxicity/pathogenicity effects of the pesticide {OPPTS 885.3050}. No clinical signs, nor abnormalities were noted during the study, and AF36 was not pathogenic nor infective following oral administration of a single dose. The LD<sub>50</sub> is greater than 5000 mg/kg when administered orally. Acute pulmonary infectivity/testing indicate no infectivity/pathogenicity effects of the pesticide {OPPTS 885.3150}. Clearance patterns were detected 8 days following intratracheal administration of  $1.93 - 2.90 \times 10^8$  cfu/rat of the pesticide, without Tween 80. There were no treatment-related mortalities, and the active ingredient was considered not infective or not pathogenic by the pulmonary route. Based on results of these mammalian tests, the pesticide was classified as Toxicity Categories IV and III, respectively for acute oral and pulmonary effects.

A rationale for waiving further testing under OPPTS Guideline 885.4150 was submitted by the applicants: AF36 is a naturally occurring strain of *Aspergillus flavus*, and a ubiquitous saprophyte commonly found in soil and plant material. Furthermore, actual field use under a multiple year EUP (69224-EUP-1) resulted in no reports of adverse effects to terrestrial mammals.

#### **c. Aquatic Species hazard assessment**

Freshwater organisms exposure to *Aspergillus flavus* AF36 is considered likely if cotton fields are adjacent to a freshwater source. However, in consideration of the natural population fluctuations of *A. flavus*, the intended use pattern, and data from soil and air population monitoring [MRID #s 45307201, 45307202; BPPD Review, May 15, 2003], such incremental exposures of AF36 would not present a hazard to aquatic organisms.

Estuarine and marine exposures to AF36 are considered less likely than their freshwater relatives. In addition to the MPCA's indigenous presence in the ecosystem, the waiver rationale claimed: a) that minimal exposures and runoff or drift to aquatic ecosystems will occur because of directed application to cotton soils with a granular formulation and no reports of *A. flavus* pathogenesis to aquatic organisms.

Accordingly, all toxicity/pathogenicity studies for aquatic organisms are waived: freshwater fish {OPPTS 885.4200}, freshwater aquatic invertebrates {OPPTS 885.4240}, and estuarine and marine organisms {OPPTS 885.4280}.

#### **d. Terrestrial and Aquatic Plants hazard assessment**

Results of soil and air monitoring studies showed that a one time seasonal application of AF36 does not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations [MRID#s 45307201, 45307202; BPPD Review, May 15, 2003]. Although the Agency waived pathogenicity testing to nontarget plants [BPPD Review, April 24, 1996; Review, June 23, 1999], the Applicants formally submitted the rationale which asserts that the MPCA is a naturally occurring strain of *Aspergillus flavus*, a ubiquitous saprophyte commonly found in soil and plant tissues.

#### **e. Terrestrial Insects hazard assessment**

Results of soil and air monitoring studies showed that a one time seasonal application of AF36 does not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations [MRID#s 45307201, 45307202; BPPD Review, May 15, 2003]. Also, the exposure and potential hazard of *A. flavus*-colonized wheat seed to foraging honey bees (*Apis mellifera* L.) on blooming cotton was assessed for 30 days, following an aerial application at label rates. *Aspergillus flavus* AF36 applied once at 10 lbs/acre was not hazardous to honey bees [MRID 45739102; DER from Alan H. Reynolds, April 29, 2003]. Therefore, no incremental hazards of AF36 are anticipated for resident non-target insects.

The Agency waived pathogenicity testing to nontarget insects [Review, June 23, 1999], and requested that the Applicants formally submit a rationale to waive pathogenicity testing to insects, except honeybees. The acceptable rationale asserts that the MPCA is a naturally occurring strain of *Aspergillus flavus*, a ubiquitous saprophyte commonly found in soil and plant tissues, and that actual field use under an EUP resulted in no reports of adverse effects to insects.

#### **f. Honeybee Hazard Assessment**

In 1999, EPA determined that a honeybee pathogenicity test be performed to assess pathogenicity of AF36 [Review, June 23, 1999], because *A. flavus* is a known, but infrequent pathogen to honeybees and other bee species causing stonebrood in the hives.

The exposure and potential hazard of AF36 colonized-wheat seed to honey bees (*Apis mellifera* L.) was assessed for 30 days following a single aerial application on blooming cotton in Arizona [MRID 45739102; DER from Alan H. Reynolds, April 29, 2003]. The control and test plots of healthy cotton (*Gossypium* spp.) grown under standard agronomic practices were each approximately 40 acres and were separated by approximately 1/2 mile. The number of dead bees for the colonies in the treated cotton plots was always less than 100 per colony per day, which is considered to be normal die off and non-hazardous. Very few dead immature bees were observed in the treated (8 total) or control plots (24 total) over the 24 observation dates. *Aspergillus flavus* AF36 applied once at 10 lbs/acre was not hazardous to honey bees, based on a pesticide hazard rating system. This field study is compliant with Good Laboratory Practices of the U. S. EPA Federal Insecticide, Fungicide and Rodenticide Acts (FIFRA), Toxic Substances Control Act (TSCA), and is consistent with Good Laboratory Practices of the Organization for Economic and Cooperative Development (OECD) and Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan.

Furthermore, results of soil and air monitoring studies showed that a one time seasonal application of AF36 does not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations [MRID#s 45307201, 45307202; BPPD Review, May 15, 2003].

#### **C. Endangered Species Assessment**

The Agency addressed potential hazards to endangered species, primarily avian species during the EUP. Also, the Registrant submitted an evaluation of plover biology and habitat [MRID

45739103; BPPD Review of Study: "Aspergillus flavus isolate AF36: Non-target Organism and Environmental Safety Information, Amendment Number 1 to MRID 45307202" Gail Tomimatsu, April 22, 2003], accompanied by a Federal Register Notice from the U.S. Fish and Wildlife Service, designating critical habitats for wintering populations of the piping plover [Federal Register, July 10, 2001 Vol. 66, No. 132, Pages 36038-36077].

No incremental hazards of AF36 are anticipated to endangered avian species in Arizona and Texas, due to results from the avian inhalation test [MRID 45798102; DER from Gail Tomimatsu, April 16, 2003], and assessments of feeding habits and preferred habitats for birds of prey and insectivorous birds in Arizona, and for plovers in Texas. For example, birds of prey such as the Bald Eagle, Cactus Ferruginous Pygmy Owl, Mexican Spotted Owl, Northern Aplomado Falcon, and the Peregrine Falcon in Arizona do not feed on wheat seed. Likewise, carnivorous species such as the Southwestern flycatcher and Yuma Clapper Rail prefer terrestrial insects [Review, June 23, 1999].

Plovers are migratory, carnivorous birds which overwinter in the Coastal Bend region of Texas during the summer months. Sightings of some species are rare in late May, June and early July. Seasonal checklists of Piping Plover and Snowy Plover have shown that these species are essentially absent at the time proposed for AF36 treatment. These birds prefer aquatic-based invertebrates and insects, indigenous among moist shorelines and algal mats, not found in agricultural lands (BPPD Review, April 22, 2003).

Likewise no incremental hazards of AF36 are anticipated to endangered mammals due to results from acute oral pathogenicity and acute pulmonary pathogenicity tests. The pesticide is applied one time per year at a low rate; less than 0.1 g a.i. per acre, usually during late spring (late May - early June). In consideration of the evaluation of endangered species' biology, and summary of the environmental expression and ecological effects to date, no hazards are anticipated for endangered wildlife.

### **3. Ecological Exposure and Environmental Expression Hazard Characterization**

In summary, it is anticipated that a one time seasonal application of AF36 should not appreciably change the overall quantity of *A. flavus* spore load on the environment, within the range of natural variations. Incremental exposures of AF36 to the environment and to nontarget organisms which inhabit or pass through the treated cotton agroecosystems do not present an adverse concern as a consequence of this registered use.

The ecological test and environmental expression data support a conclusion of reasonable certainty that no incremental hazards to nontarget organisms, or to the environment are anticipated as a result of the intended use. No further testing for ecological effects is necessary for this conditional registration. However, for environmental expression in other cotton-growing states or regions, e.g., Texas, additional testing or research may be necessary to satisfy concerns for product performance, or efficacy in reducing aflatoxin levels in cottonseed.

## References:

1. Tomimatsu, G. S. and Robert H. Rose. 1996. Memorandum to Shanaz Bacchus. BPPD Review of Information Submitted by USDA Southern Regional Research Center/IR-4 for an Experimental Use Permit for *Aspergillus flavus* AF36; Request for Waiver for Non-Target Plant Testing (DP Barcode No: D224186, Chemical ID No.: 006456)
2. Gurian-Sherman, D. and G. S. Tomimatsu. 1999. Memorandum to Shanaz Bacchus. Review of Request for Waiver of Non-Target Organism Safety Effects Tests for EUP Renewal and Expansion for Biopesticide Containing *Aspergillus flavus* AF36 (DP Barcode: D248828)
3. Tomimatsu, G.S. and Z. Vaituzis. 2001. Review of Protocol for Testing the Toxicity/Pathogenicity of the MPCA, *Aspergillus flavus* Strain AF36 (Chemical No.: 006456) to Avian Species (DP Barcode D274694).
4. Tomimatsu, G.S., and J. Kough. 2003. BPPD Review of Soil and Air Monitoring Studies and Product Performance Testing (Efficacy) Submitted by USDA Southern Regional Research Center/IR-4 as a Condition of Registration and EUP Extension (Texas) for *Aspergillus flavus* AF36 (Submission #: S630862; DP Barcode D288777; ID# 071693-R, Chemical No. 006456) [MRID #s 453072-01; 453072-02; 1 volume sans MRID]
5. Tomimatsu, G.S. 2003. BPPD Review of Study: "Aspergillus flavus isolate AF36: Non-target Organism and Environmental Safety Information, Amendment Number 1 to MRID 45307202. April 22, 2003.

DP BARCODE: 1288782

CASE: 062458  
SUBMISSION: S630867DATA PACKAGE RECORD  
BEAN SHEETDATE: 03/11/03  
Page 1 of 1

## \* \* \* CASE/SUBMISSION INFORMATION \* \* \*

CASE TYPE: REGISTRATION ACTION: 157 DATA WAIV REQ-NEW BIOL  
CHEMICALS: 006456 Aspergillus flavus 36 colonized wheat seed 0.0000%ID#: 071693-R Aspergillus Flavus AF36  
COMPANY: 071693 ARIZONA COTTON RESEARCH AND PROTECTION COUNCIL  
PRODUCT MANAGER: 90 JANET ANDERSEN 703-308-8128 ROOM: CS1 5TH FL  
PM TEAM REVIEWER: SHANAZ BACCHUS 703-308-8097 ROOM: CS1 5TH FL  
RECEIVED DATE: 3/11/03 DUE OUT DATE: 08/08/03

## \* \* \* DATA PACKAGE INFORMATION \* \* \*

DP BARCODE: 288782 EXPEDITE: N DATE SENT: 03/11/03 DATE RET.: / /  
CHEMICAL: 006456 Aspergillus flavus 36 colonized wheat seed  
DP TYPE: 001

CSF: Y	LABEL: Y		
ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 06/24/03
DIV : BPPD	03/11/03	/ /	NEGOT DATE: / /
BRAN: BPPD-IO	03/11/03	/ /	PROJ DATE: / /
SECT: IO	03/11/03	/ /	
REVR : GTOMIMAT	03/11/03	/ /	
CONTR:	/ /	/ /	

## \* \* \* DATA REVIEW INSTRUCTIONS \* \* \*

Please determine whether the justifications for waiving ecological effects data are acceptable (Section 2 of this submission). If there are deficiencies in the justifications, please state what steps/further action the company needs to take to fulfil data requirements.

Thanks,  
shawn

## \* \* \* DATA PACKAGE EVALUATION \* \* \*

No evaluation is written for this data package

## \* \* \* ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION \* \* \*

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
-------	----------------	----------	----------	-----	-----	-------



13544



R141748

Chemical: *Aspergillus flavus* 36 colonized wheat seed

PC Code:  
006456

HED File Code: 41300 BPPD Eco Effects

Memo Date: 5/16/2003

File ID: DPD288777

DPD288782

DPD286708

Accession #: 000-00-9002

HED Records Reference Center  
4/13/2007

